

AMENDMENTS TO THE CLAIMS:

Please amend the claims as follows:

1. (currently amended) A modified bacterial surface layer (S-layer) protein, the modification comprising the internal insertion of a heterologous polypeptide, wherein said unmodified protein is ~~able to crystallize to form a crystalline monolayer and the unmodified protein is from a gram-positive bacterium;~~

(a) able to crystallize to form a crystalline monolayer;

(b) from a *Lactobacillus* bacterium;

(c) from 40 to 70 kd in size; and

(d) highly basic with a pI of at least 9.

where the insertion site of said heterologous polypeptide is:

(i) at a position from amino acids 1 to 20;

(ii) at a position from amino acids 35 to 55;

(iii) at a position from amino acids 100 to 130;

(iv) at a position from amino acids 110 to 140;

(v) at the position of amino acid 193; and/or

(vi) at a position from amino acids 340 to 360.

2. (previously amended) A protein according to claim 1 wherein the heterologous polypeptide is a functional polypeptide or a polypeptide of interest.

3. (currently amended) A protein according to claim 1 wherein[[:]]

[[[c)]] the protein retains most of the full length sequence of the unmodified S-layer protein[[:]]

(d) ~~the polypeptide is inserted at an internal location at least five amino acids from the C or N terminus; and/or~~

(e) ~~the modified protein has a size of from 40 to 300 kDa.~~

4. (currently amended) A protein according to claim 1 which:

[[f)] (e) has a crystallisation or N-terminal domain that is predominantly basic, or hydrophobic,

[[g)] (f) has a [[an]] C-terminal domain which is predominantly basic or hydrophilic; or

[[h)] (g) has alternating hydrophobic and hydrophilic regions.

5. (currently amended) A protein according to claim 1 wherein the heterologous polypeptide is inserted at a location in the protein either so that it is:

[[i)] (h) exposed, or present on the cell surface;

[[j)] (i) present in the surface layer, or the cell wall; or

[[k)] (j) is protected from external proteolytic processing or is not recognised or bound by external antibodies.

6. (currently amended) A protein according to claim 1 wherein the modified or unmodified protein:

[[l)] (k) crystallizes into an oblique lattice; and/or

[[m)] (l) has a cell wall anchor domain[[:]]

(n) ~~has a pI of at least 7; and/or~~

(o) ~~is predominantly basic.~~

Claim 7. (cancelled)

8. (previously amended) A protein according to claim 1 which in unmodified form is from *Lactobacillus acidophilus*, *L. crispatus*, *L. helveticus*, *L. amylovorus*, or *L. gallinarum*.

9. (withdrawn) A fragment of a protein according to claim 1 which is:

a) an N-terminal fragment or a fragment that is capable of forming a dimer with another such fragment or a trimer with two other such fragments;

b) capable of forming dimers with another such fragment and either

(i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or

(ii) excludes an entire immunodominant or exposed loop region and is from 20 to 155 amino acids long.

10. (withdrawn) A polynucleotide encoding a protein according to claim 1.

11. (withdrawn) A vector comprising a polynucleotide according to claim 10.

12. (withdrawn) A host cell comprising, or which has been transformed with a vector according to claim 11.

13. (withdrawn) A bacteria expressing a surface layer (S-layer) protein according to claim 1 or a fragment thereof which is:

a) an N-terminal fragment or a fragment that is capable of forming a dimer with another such fragment or a trimer with two other such fragments;

b) capable of forming dimers with another such fragment and either

(i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or

(ii) excludes an entire immunodominant loop region and is from 20 to 155 amino acids long.

14. (withdrawn) A bacteria according to claim 13 which is a lactic acid bacteria.

15. (withdrawn) A modified bacteria which has been modified to express a heterologous surface layer (S-layer) protein, wherein said heterologous surface layer (S-layer) protein is a modified bacterial surface layer (S-layer) protein according to claim 1.

16. (withdrawn) A bacteria according to claim 15 which would not normally, or as a wild-type or in unmodified form does not, possess a surface layer.

17. (withdrawn) A modified bacteria according to claim 15 which is a *Lactobacillus* cell and/or the S-layer has its own, original, cell wall anchor.

18. (withdrawn) A bacteria according to claim 15 which is a *Lactobacillus* bacterial cell and/or the S-layer protein is from *Lactobacillus* bacteria.

Claims 19-20 (Cancelled)

21. (withdrawn) A modified bacteria expressing only, or homogeneously, a modified surface layer (S-layer) protein according to claim 1.

22. (withdrawn) A bacteria according to claim 21 having a genome which includes a polynucleotide encoding a modified S-layer protein, and/or where the polynucleotide encoding the normal or wild-type S-layer protein has been silenced, replaced, switched off or otherwise rendered non-expressed.

23. (withdrawn) A bacteria according to claim 22 wherein the modified S-layer protein is the sole or only S-layer protein expressed by the bacterial cell and/or the cell does not express any wild-type S-layer protein.

24. (withdrawn) A bacteria according to claim 22 wherein the S-layer protein is located on the surface of the cell wall and/or a multiplicity of S-layer proteins form an S-layer.

25. (withdrawn) A vaccine comprising a bacteria according to claim 13, a modified bacteria which has been modified to express a heterologous surface layer (S-layer) protein, wherein said heterologous surface layer (S-layer) protein is a modified bacterial surface layer (S-layer) or a modified bacteria expressing only, or homogeneously, a modified surface layer (S-layer) protein, wherein said bacteria has GRAS (generally regarded as safe) status.

26. (withdrawn) A vaccine according to claim 25 which is an oral or nasal vaccine and/or additionally comprises an adjuvant.

27. (withdrawn) A sheet or monolayer or 2-dimensional array comprising a plurality of bacterial surface layer proteins, at least one of which is modified protein according to claim 1.

28. (withdrawn) A solid surface, liquid-air interface, lipid film, liposome or solution comprising a sheet, monolayer or array according to claim 27.

29. (withdrawn) A solid surface according to claim 28 to which is bound one or more (macro) molecules, such as an enzyme, antibody or other binding molecule, receptor, antigen or ligand.

30. (withdrawn) A solid surface comprising a layer of S-proteins, at least a plurality of which are modified proteins according to claim 1, sandwiched between the surface and a layer of functional molecules.

31. (withdrawn) A sensor, molecular sieve or ion trap comprising a sheet, layer or array according to claim 27 or a surface comprising a sheet, monolayer or array.

32. (withdrawn) A sensor, molecular sieve or ion trap comprising a solid surface comprising a layer of S-proteins, at least a plurality of which are modified proteins according to claim 1, sandwiched between the surface and a layer of functional molecules.

Claim 33. (canceled)

34. (currently amended) A protein according to claim 8 wherein the modified ~~unmodified~~ protein is from *Lactobacillus acidophilus* ~~and wherein the heterologous polypeptide is inserted at position 1 to 290 of SEQ ID NO: 2.~~

35. (withdrawn) A protein according to claim 34 wherein the heterologous polypeptide is inserted at a location:

- (i) from amino acid 1 to amino acid 20 of SEQ ID NO: 2;
- (ii) from amino acid 35 to amino acid 55 of SEQ ID NO: 2;
- (iii) from amino acid 100 to amino acid 130 of SEQ ID NO: 2; and/or
- (iv) from amino acid 110 to amino acid 140 of SEQ ID NO: 2.

36. (withdrawn) A protein according to claim 35 wherein the heterologous polypeptide is inserted at a location:

- (i) from amino acid 5 to amino acid 10 of SEQ ID NO: 2;

- (ii) from amino acid 40 to amino acid 50 of SEQ ID NO: 2;
- (iii) from amino acid 110 to amino acid 120 of SEQ ID NO: 2; and/or
- (iv) from amino acid 120 to amino acid 130 of SEQ ID NO: 2.

37. (withdrawn) A protein according to claim 36 wherein the heterologous polypeptide is inserted at position 7, 45, 114 and/or 125 of SEQ ID NO: 2.

38. (withdrawn) A method of using a protein according to claim 1, said method comprising administering the modified protein or a bacteria expressing the modified protein to a human or animal by mucosal, nasal, oral, or vaginal delivery.

39. (withdrawn) A method of making a protein according to claim 1, said method comprising cultivating a host cell under conditions to provide for expression of the protein and recovering the expressed protein.

40. (New) A modified bacterial surface layer (S-layer) protein, the modification comprising the internal insertion of a heterologous polypeptide, wherein said unmodified protein is:

- (a) able to crystallize to form a crystalline monolayer *in vitro*;
- (b) from a *Lactobacillus* bacterium;
- (c) from 40 to 70 kd in size; and
- (d) highly basic with a pI of at least 9;

where the insertion site of said heterologous polypeptide is:

- (i) at a position from amino acids 1 to 20;
- (ii) at a position from amino acids 35 to 55;
- (iii) at a position from amino acids 100 to 130;

- (iv) at a position from amino acids 110 to 140;
- (v) at the position of amino acid 193; and/or
- (vi) at a position from amino acids from amino acids 290 to 410.